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UTILITY PATENT APPLICATION TRANSMITTAL

Only for new nonprovisional applications under 37 CFR 1.53(b)

Attorney Docket No. BB1162 US NA

First Named Inventor or Application Identifier

STEPHEN M. ALLEN et al.

Express Mail Label No. EL073740966US

Express Mailing Date October 5, 2000

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09/27/00
U.S. PTO

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO:

Assistant Commissioner for Patents
Box Patent Application
Washington, DC 202311. ☒ Fee (Authority to charge deposit account below.)
(Submit an original, and a duplicate for fee processing)2. ☒ Specification [Total Pages 27]

- Descriptive title of the invention
- Cross References to Related Applications (if needed)
- Statement Regarding Fed sponsored R & D (if needed)
- Reference to Microfiche Appendix (if filed)
- Background of the Invention
- Brief Summary of the Invention

- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) (35 USC 113)

[Total Sheets 6]

4. ☐ Oath or Declaration

[Total Pages 0]

a. ☐ Newly executed (original or copy)b. ☐ Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 14 completed)i. ☐ DELETION OF INVENTORSSigned Statement below at 15 deleting
inventor(s) named in the prior application,
see 37 CFR 1.63(d)(2) and 1.33(b).
☐ Incorporation by Reference (useable if Box 4b is checked)
The entire disclosure of the prior application, from which a
copy of the oath or declaration is supplied under Box 4b, is
considered as being part of the disclosure of the
accompanying application and is hereby incorporated by
reference therein.
6. ☐ Microfiche Computer Program (Appendix)7. Nucleotide and/or Amino Acid Sequence Submission
(if applicable, all necessary)a. ☒ Computer Readable Copyb. ☒ Paper Copy (identical to computer copy)
Sequence Listing - 30 pgs.c. ☒ Statement verifying identity of above copiesDeclaration in Accordance with
37 CFR 1.821

ACCOMPANYING APPLICATION PARTS

8. ☒ Power of Attorney9. ☐ Information Disclosure
Statement (IDS)/Cover ☐ Copies of IDS
Letter plus PTO-1449 Citations10. ☒ Preliminary Amendment11. ☒ Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)12. ☐ Certified Copy of Priority Document(s)
(if foreign priority is claimed)13. ☐ Other:

14. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:

☒ Continuation☐ Divisional☐ Continuation-in-part (CIP)

of prior Application No.: PCT/US99/07562

15. ☐ DELETION OF INVENTOR(S) STATEMENT: This application is being filed by less than all the inventors named in the prior application. In accordance with 37 CFR 1.63(d)(2) and 1.33(b), the Assistant Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application:16. ☐ Amend the specification by inserting before the first line the sentence:-- This is a ☐ continuation-in-part, ☐ continuation, ☐ division of Application No. _____ filed
, now abandoned. --17. ☐ Cancel in this application original claims ____ of the prior application before calculating the filing. (At least one original independent claim must be retained for filing purposes.)18. ☐ Priority of foreign Application No. _____ filed on _____ in __________ is claimed under 35 U.S.C. 119.
(country)

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS (37 CFR 1.16(c))	10 - 20 =	0	x \$ 18 =	0
	INDEPENDENT CLAIMS (37 CFR 1.16(b))	3 - 3 =	0	x \$ 80 =	0
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$ 270 =	0
				BASIC FEE (37 CFR 1.16(a))	+ \$ 710.00
				TOTAL =	\$ 710.00

19. The Commissioner is hereby authorized to credit overpayments or charge the following fees to Deposit Account No. 04-1928:

a. ☒ Fees required under 37 CFR 1.16.

b. ☒ Fees required under 37 CFR 1.17.

20. ☐ Other:

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22. SIGNATURE OF ATTORNEY OR AGENT REQUIRED

NAME	Thomas M. Rizzo	REG. NO.: 41,272
SIGNATURE	<i>Thomas M Rizzo</i>	
DATE	<i>October 5, 2000</i>	

EXPRESS MAIL LABEL NO. EL073740966US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

S. ALLEN ET AL.

CASE NO.: BB1162 US NA

APPLICATION NO.: UNKNOWN

GROUP ART UNIT: UNKNOWN

FILED: CONCURRENTLY HEREWITH

EXAMINER: UNKNOWN

FOR: SUCROSE TRANSPORTERS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Before examination of the above-referenced application, please amend the application as follows:

IN THE SPECIFICATION

On page 1, lines 3 and 4, replace the sentence with:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of International Application No. PCT/US 99/07562, filed April 7, 1999, now pending, which claims priority benefit to U.S. Provisional Application No. 60/081,148 filed April 9, 1998.--

IN THE CLAIMS

Cancel claims 1-10.

Add the following claims:

11. An isolated polynucleotide comprising:

(a) a nucleotide sequence encoding a polypeptide, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24 have at least 95% identity based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence.

12. The polynucleotide of Claim 11, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID

NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24 have at least 90% identity based on the Clustal alignment method.

13. The polynucleotide of claim 11 comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, or SEQ ID NO:23.

14. The polynucleotide of claim 11, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24.

15. The polynucleotide of claim 11, wherein the polypeptide is a sucrose transport protein.

16. An isolated polypeptide, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24 have at least 95% identity based on the Clustal alignment method.

17. The polypeptide of Claim 16, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24 have at least 90% identity based on the Clustal alignment method.

18. The polypeptide of claim 16, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24.

19. The polypeptide of claim 16, wherein the polypeptide is a sucrose transport protein.

20. A chimeric gene comprising the polynucleotide of claim 11 operably linked to a regulatory sequence.

21. An expression vector comprising the polynucleotide of claim 11.
22. A method for transforming a cell comprising transforming a cell with the polynucleotide of claim 11.
23. The cell produced by the method of claim 22.
24. An isolated polynucleotide comprising a nucleotide sequence comprised by the polynucleotide of claim 11, wherein the nucleotide sequence contains at least 30 nucleotides.

REMARKS

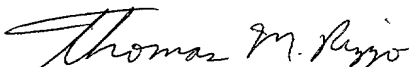
Claims 1-10 have been cancelled, and claims 11-24 have been added. Claims 11-24 are pending. It is respectfully requested that the amendments above be entered before examination of the application.

Support for sequence identities of 90% and 95% is found on page 5, lines 23-26 of the specification. Support for claim 26 is found on page 6, lines 4-8 of the specification.

Please charge the necessary fees to Deposit Account 04-1928 (E. I. du Pont de Nemours and Company). If the fee is insufficient or incorrect, please charge or credit the balance to the above-identified account.

In view of the foregoing, allowance of the above-referenced application is respectfully requested.

Respectfully submitted,



THOMAS M. RIZZO
ATTORNEY FOR APPLICANTS
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Dated: October 5, 2000

TITLE

SUCROSE TRANSPORTERS

This application claims the benefit of U.S. Provisional Application No. 60/081,148, filed April 9, 1998.

5 FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding sucrose transport proteins in plants and seeds.

BACKGROUND OF THE INVENTION

10 Sucrose is the first form of carbohydrate to leave photosynthesizing cells in most higher plants and is the main form of transported carbon in most annual field crops plants such as corn, soybeans and wheat. As such its movement and concentration across various plant membranes is critical to plant growth and development. In addition sucrose is the main form of carbon that moves into developing seeds of soybeans, corn and wheat. This
15 movement and concentration is accomplished by the action of sucrose carrier proteins that act to move sucrose against a concentration gradient by coupling sucrose movement to the opposite vectoral movement of a proton. Specific sucrose carrier sequences from these crop plants should find use in controlling the timing and extent of phenomena such as grain fill duration that are important factors in crop yield and quality. Accordingly, the
20 availability of nucleic acid sequences encoding all or a portion of these enzymes would facilitate studies to better understand carbohydrate metabolism and function in plants, provide genetic tools for the manipulation of these biosynthetic pathways, and provide a means to control carbohydrate transport and distribution in plant cells.

SUMMARY OF THE INVENTION

25 The instant invention relates to isolated nucleic acid fragments encoding proteins involved in sucrose transport. Specifically, this invention concerns an isolated nucleic acid fragment encoding a sucrose transport protein. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding the sucrose transport protein. An additional embodiment of the instant invention pertains to a
30 polypeptide encoding all or a substantial portion of a sucrose transport protein.

 In another embodiment, the instant invention relates to a chimeric gene encoding a sucrose transport protein, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a sucrose transport protein, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in
35 production of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

 In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding a sucrose transport protein, operably

linked to suitable regulatory sequences. Expression of the chimeric gene results in production of altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the level of expression of a sucrose transport protein in a transformed host cell comprising:

a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a sucrose transport protein; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of sucrose transport protein in the transformed host cell.

An addition embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or a substantial portion of an amino acid sequence encoding a sucrose transport protein.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figure 1 shows a comparison of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 and the *Daucus carota* (SEQ ID NO:25), *Oryza sativa* (SEQ ID NO:26), *Ricinus communis* (SEQ ID NO:27) and *Vicia faba* (SEQ ID NO:28) sucrose transport protein amino acid sequences.

The following sequence descriptions and sequence listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

SEQ ID NO:1 is the nucleotide sequence comprising the entire cDNA insert in clone cepe7.pk0015.d10 encoding an entire corn sucrose transport protein.

SEQ ID NO:2 is the deduced amino acid sequence of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:1.

SEQ ID NO:3 is the nucleotide sequence comprising a portion of the cDNA insert in clone cr1n.pk0075.f5 encoding a portion of a corn sucrose transport protein.

SEQ ID NO:4 is the deduced amino acid sequence of a portion of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:3.

SEQ ID NO:5 is the nucleotide sequence comprising a portion of the cDNA insert in clone cr1n.pk0095.c10 encoding a portion of a corn sucrose transport protein.

SEQ ID NO:6 is the deduced amino acid sequence of a portion of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:5.

SEQ ID NO:7 is the nucleotide sequence comprising the entire cDNA insert in clone rlr2.pk0043.b1 encoding a portion of a rice sucrose transport protein.

5 SEQ ID NO:8 is the deduced amino acid sequence of a portion of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:7.

SEQ ID NO:9 is the nucleotide sequence comprising the entire cDNA insert in clone rls6.pk0076.e2 encoding an entire rice sucrose transport protein.

10 SEQ ID NO:10 is the deduced amino acid sequence of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:9.

SEQ ID NO:11 is the nucleotide sequence comprising the entire cDNA insert in clone sfl1.pk0001.g1 encoding an entire soybean sucrose transport protein.

SEQ ID NO:12 is the deduced amino acid sequence of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:11.

15 SEQ ID NO:13 is the nucleotide sequence comprising a contig assembled from the cDNA inserts in clones sfl1.pk0043.c7 and sdp3c.pk012.c13 encoding a portion of a soybean sucrose transport protein.

SEQ ID NO:14 is the deduced amino acid sequence of a portion of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:13.

20 SEQ ID NO:15 is the nucleotide sequence comprising a portion of the cDNA insert in clone vs1n.pk0002.h3 encoding a portion of a *Vernonia* sucrose transport protein.

SEQ ID NO:16 is the deduced amino acid sequence of a portion of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:15.

25 SEQ ID NO:17 is the nucleotide sequence comprising the entire cDNA insert in clone wle1n.pk0007.h8 encoding a portion of a wheat sucrose transport protein.

SEQ ID NO:18 is the deduced amino acid sequence of a portion of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:17.

SEQ ID NO:19 is the nucleotide sequence comprising the entire cDNA insert in clone wle1n.pk0103.c11 encoding an entire wheat sucrose transport protein.

30 SEQ ID NO:20 is the deduced amino acid sequence of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:19.

SEQ ID NO:21 is the nucleotide sequence comprising the entire cDNA insert in clone wlm24.pk0015.g11 encoding an entire wheat sucrose transport protein.

35 SEQ ID NO:22 is the deduced amino acid sequence of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:21.

SEQ ID NO:23 is the nucleotide sequence comprising the entire cDNA insert in clone wlmk1.pk0002.e11 encoding an entire wheat sucrose transport protein.

SEQ ID NO:24 is the deduced amino acid sequence of a sucrose transport protein derived from the nucleotide sequence of SEQ ID NO:23.

SEQ ID NO:25 is the amino acid sequence of a *Daucus carota* sucrose transport protein (NCBI Identifier No. gi 2969887).

5 SEQ ID NO:26 is the amino acid sequence of a *Oryza sativa* sucrose transport protein (NCBI Identifier No. gi 2723471).

SEQ ID NO:27 is the amino acid sequence of a *Ricinus communis* sucrose transport protein (NCBI Identifier No. gi 542020).

10 SEQ ID NO:28 is the amino acid sequence of a *Vicia faba* sucrose transport protein (NCBI Identifier No. gi 1935019).

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The
15 symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, an “isolated nucleic acid fragment” is a polymer of RNA or DNA that is single- or double-
20 stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA. As used herein, “contig” refers to an assemblage of overlapping nucleic acid sequences to form one contiguous nucleotide sequence. For example, several DNA sequences can be compared and aligned to identify
25 common or overlapping regions. The individual sequences can then be assembled into a single contiguous nucleotide sequence. As used herein, “substantially similar” refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence.

30 “Substantially similar” also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. “Substantially similar” also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the
35 functional properties of the resulting transcript vis-à-vis the ability to mediate alteration of gene expression by antisense or co-suppression technology or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary sequences.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a gene which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded protein, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the nucleic acid fragments disclosed herein.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent similarity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Preferred are those nucleic acid fragments whose nucleotide sequences encode amino acid sequences that are 90% similar to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% similar to the amino acid sequences reported herein. Sequence alignments and percent similarity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins, D. G. and Sharp, P. M. (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10), (hereafter Clustal algorithm). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to afford putative identification of that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool;

Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also

www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches partial or complete amino acid and nucleotide sequences encoding one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the sucrose transport proteins as set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments which are then enzymatically assembled to construct the entire gene. "Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased

towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature.

“Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a DNA sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg, (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G. D. (1995) *Molecular Biotechnology* 3:225).

The “3' non-coding sequences” refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into protein by the cell. “cDNA” refers to a double-stranded DNA that is complementary to and derived from mRNA. “Sense” RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. “Antisense RNA” refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term “operably linked” refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. “Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. “Overexpression” refers to the production

of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

5 "Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed.

10 "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a
15 nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels, J. J., (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention
20 signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the
25 transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

30 Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

35 Nucleic acid fragments encoding at least a portion of several sucrose transport proteins have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. Table 1 lists the proteins that are described herein, and the

designation of the cDNA clones that comprise the nucleic acid fragments encoding these proteins.

TABLE 1

Sucrose Transport Proteins

Enzyme	Clone	Plant
Sucrose Transporter	cepe7.pk0015.d10	Corn
	cr1n.pk0095.c10	Corn
	cr1n.pk0075.f5	Corn
	rlr2.pk0043.b1	Rice
	rls6.pk0076.e2	Rice
	sfl1.pk0001.g1	Soybean
	sfl1.pk0043.c7	Soybean
	sdp3c.pk012.c13	Soybean
	vs1n.pk0002.h3	Vernonia
	wle1n.pk0007.h8	Wheat
	wle1n.pk0103.c11	Wheat
	wlm24.pk0015.g11	Wheat
	wlmk1.pk0002.e11	Wheat

The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other sucrose transport proteins, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during

amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al., (1988) *PNAS USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al., (1989) *PNAS USA* 86:5673; Loh et al., (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman, M. A. and Martin, G. R., (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner, R. A. (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed sucrose transport proteins are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of sucrose metabolism in those cells.

Overexpression of the sucrose transport proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) *EMBO J.* 4:2411-2418; De Almeida et al., (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant sucrose transport proteins to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode a sucrose transport protein with appropriate intracellular targeting sequences such as transit sequences (Keegstra, K. (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels, J.J., (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel, N. (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding sucrose transport proteins in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant sucrose transport proteins can be constructed by linking a gene or gene fragment encoding a sucrose transport protein to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

The instant sucrose transport proteins (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting sucrose transport proteins *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant sucrose transport proteins are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene

for production of the instant sucrose transport proteins. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded sucrose transport protein. An example of a vector for high level expression of the instant sucrose transport proteins in a bacterial host is provided

(Example 6).

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al., (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein, D. et al., (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in R. Bernatzky, R. and Tanksley, S. D. (1986) *Plant Mol. Biol. Reporter* 4(1):37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel, J. D., et al., In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask, B. J. (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan, M. et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian, H. H. (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield, V. C. et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren, U. et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov, B. P. (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter, M. A. et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping (Dear, P. H. and Cook, P. R. (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer, (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al., (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al., (1995) *Plant Cell* 7:75). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the sucrose transport protein. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding a sucrose transport protein can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the sucrose transport protein gene product.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without

departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

- 5 cDNA libraries representing mRNAs from various corn, rice, soybean, *Vernonia* and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn Rice, Soybean *Vernonia* and Wheat

Library	Tissue	Clone
cepe7	Corn epicotyl from 7 day old etiolated seedling	cepe7.pk0015.d10
cr1n	Corn root from 7 day seedling grown in light *	cr1n.pk0075.f5 cr1n.pk0095.c10
rlr2	Rice leaf 15 days after germination 2 hours after infection of strain <i>Magnaporthe grisea</i> 4360-R-62 (AVR2-YAMO)	rlr2.pk0043.b1
rls6	Rice leaf 15 days after germination 6 hours after infection of strain <i>Magnaporthe grisea</i> 4360-R-62 (AVR2-YAMO)	rls6.pk0076.e2
sdp3c	Soybean developing pods 8-9 mm	sdp3c.pk012.c13
sfl1	Soybean immature flower	sfl1.pk0001.g1 sfl1.pk0043.c7
vs1	Vernonia developing seed	vs1n.pk0002.h3
wle1n	Wheat leaf 7 day old etiolated seedling light grown*	wle1n.pk0007.h8 wle1n.pk0103.c11
wlm24	Wheat seedling 24 hours after inoculation with <i>Erysiphe graminis</i>	wlm24.pk0015.g11
wlmk1	Wheat seedlings 1 hour after inoculation with <i>Erysiphe graminis</i> and treatment with fungicide**	wlmk1.pk0002.e11

- 10 *These libraries were normalized essentially as described in U.S. Patent No. 5,482,845
 **Application of 6-iodo-2-propoxy-3-propyl-4(3*H*)-quinazolinone; synthesis and methods of using this compound are described in USSN 08/545,827, incorporated herein by reference.

- 15 cDNA libraries were prepared in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). Conversion of the Uni-ZAP™ XR libraries into plasmid libraries was accomplished according to the protocol provided by Stratagene. Upon conversion, cDNA inserts were contained in the plasmid vector pBluescript. cDNA inserts from randomly picked bacterial colonies containing
 20 recombinant pBluescript plasmids were amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences or plasmid DNA was prepared from cultured bacterial cells. Amplified insert DNAs or plasmid DNAs

were sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or “ESTs”; see Adams, M. D. et al., (1991) *Science* 252:1651). The resulting ESTs were analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

ESTs encoding sucrose transport proteins were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST “nr” database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the “nr” database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the “nr” database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272 and Altschul, Stephen F., et al. (1997) *Nucleic Acids Res.* 25:3389-3402) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as “pLog” values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST “hit” represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Sucrose Transporter Proteins

The BLASTX search using the EST sequences from clones cepe7.pk0015.d10, cr1n.pk0095.c10, cr1n.pk0075.f5, rls6.pk0076.e2, wle1n.pk0007.h8, wle1n.pk0007.h8, wle1n.pk0103.c11, wlm24.pk0015.g11 and wlmk1.pk0002.e11 revealed similarity of the proteins encoded by the cDNAs to a sucrose transporter from *Oryza sativa* (NCBI Identifier No. gi 2723471). The BLASTX search using the EST sequence from clone rlr2.pk0043.b1 revealed similarity of the protein encoded by the cDNA to a sucrose transporter from *Daucus carota* (NCBI Identifier No. gi 2969887). The BLASTX search using the EST sequence from clone sfl1.pk0001.g1 revealed similarity of the protein encoded by the cDNA to a sucrose transporter from *Vicia faba* (NCBI Identifier No. gi 1935019). The BLASTX search using the EST sequences from clones sfl1.pk0043.c7, sdp3c.pk012.c13 and vs1n.pk0002.h3 revealed similarity of the proteins encoded by the cDNAs to a sucrose transporter from *Ricinus communis* (NCBI Identifier No. gi 542020).

In the process of comparing the ESTs it was found that soybean clones sfl1.pk0043.c7 and sdp3c.pk012.c13 had overlapping regions of homology. Using this homology it was possible to align the ESTs and assemble a contig encoding a unique soybean sucrose transport protein.

- 5 The BLAST results for each of these ESTs and the soybean contig are shown in Table 3:

TABLE 3

10 BLAST Results for Clones Encoding Polypeptides Homologous to
Daucus carota, *Oryza sativa*, *Ricinus communis* and *Vicia faba* Sucrose Transport Proteins

Clone	BLAST pLog Score
cepe7.pk0015.d10	>250.00
cr1n.pk0095.c10	>250.00
cr1n.pk0075.f5	31.10
rlr2.pk0043.b1	148.00
rls6.pk0076.e2	>250.00
sfl1.pk0001.g1	>250.00
Contig composed of: sfl1.pk0043.c7 sdp3c.pk012.c13	142.00
vs1n.pk0002.h3	59.30
wle1n.pk0007.h8	110.00
wle1n.pk0103.c11	>250.00
wlm24.pk0015.g11	>250.00
wlmk1.pk0002.e11	177.00

- 15 The sequence of a portion of the cDNA insert from clone cepe7.pk0015.d10 is shown in SEQ ID NO:1; the deduced amino acid sequence of this cDNA, which represents 100% of the protein, is shown in SEQ ID NO:2. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:2 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:2 is 82% similar to the *Oryza sativa* protein.

- 20 The sequence of a portion of the cDNA insert from clone cr1n.pk0075.f5 is shown in SEQ ID NO:3; the deduced amino acid sequence of this cDNA, which represents 93% of the protein, is shown in SEQ ID NO:4. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:4 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:4 is 50% similar to the *Oryza sativa* protein.

The sequence of a portion of the cDNA insert from clone cr1n.pk0095.c10 is shown in SEQ ID NO:5; the deduced amino acid sequence of this cDNA, which represents 20% of the protein (C-terminal region), is shown in SEQ ID NO:6. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:6 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:6 is 86% similar to the *Oryza sativa* protein.

The sequence of a portion of the cDNA insert from clone rlr2.pk0043.b1 is shown in SEQ ID NO:7; the deduced amino acid sequence of this cDNA, which represents 79% of the protein (C-terminal region), is shown in SEQ ID NO:8. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:8 and the *Daucus carota* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:8 is 60% similar to the *Daucus carota* protein.

The sequence of a portion of the cDNA insert from clone rls6.pk0076.e2 is shown in SEQ ID NO:9; the deduced amino acid sequence of this cDNA, which represents 100% of the protein, is shown in SEQ ID NO:10. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:10 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:10 is 55% similar to the *Oryza sativa* protein. Due to a percent similarity of only 55% with a known rice sucrose transport protein clone rls6.pk0076.e2 appears to represent a second rice sucrose transport protein.

The sequence of a portion of the cDNA insert from clone sfl1.pk0001.g1 is shown in SEQ ID NO:11; the deduced amino acid sequence of this cDNA, which represents 100% of the protein, is shown in SEQ ID NO:12. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:12 and the *Vicia faba* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:12 is 67% similar to the *Vicia faba* protein.

The sequence of a portion of the contig composed of clones sfl1.pk0043.c7 and sdp3c.pk012.c13 is shown in SEQ ID NO:13; the deduced amino acid sequence of this contig, which represents 62% of the protein (N-terminal region), is shown in SEQ ID NO:14. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:14 and the *Ricinus communis* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:14 is 66% similar to the *Ricinus communis* protein.

The sequence of a portion of the cDNA insert from clone vs1n.pk0002.h3 is shown in SEQ ID NO:15; the deduced amino acid sequence of this cDNA, which represents 31% of the protein (C-terminal region), is shown in SEQ ID NO:16. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:16 and the *Ricinus communis* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:16 is 66% similar to the *Ricinus communis* protein.

The sequence of a portion of the cDNA insert from clone wle1n.pk0007.h8 is shown in SEQ ID NO:17; the deduced amino acid sequence of this cDNA, which represents 43% of the protein (C-terminal region), is shown in SEQ ID NO:18. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:18 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:18 is 80% similar to the *Oryza sativa* protein.

The sequence of a portion of the cDNA insert from clone wle1n.pk0103.c11 is shown in SEQ ID NO:19; the deduced amino acid sequence of this cDNA, which represents 100% of the protein, is shown in SEQ ID NO:20. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:20 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:20 is 80% similar to the *Oryza sativa* protein.

The sequence of a portion of the cDNA insert from clone wlm24.pk0015.g11 is shown in SEQ ID NO:21; the deduced amino acid sequence of this cDNA, which represents 100% of the protein, is shown in SEQ ID NO:22. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:22 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:22 is 80% similar to the *Oryza sativa* protein.

The sequence of a portion of the cDNA insert from clone wlmk1.pk0002.e11 is shown in SEQ ID NO:23; the deduced amino acid sequence of this cDNA, which represents 97% of the protein, is shown in SEQ ID NO:24. A calculation of the percent similarity of the amino acid sequence set forth in SEQ ID NO:24 and the *Oryza sativa* sequence (using the Clustal algorithm) revealed that the protein encoded by SEQ ID NO:24 is 54% similar to the *Oryza sativa* protein.

The percent similarity between each of the corn, rice, soybean, *Vernonia* and wheat amino acid sequence was calculated to range from 12 to 98% using the Clustal algorithm. Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 and the *Daucus carota*, *Oryza sativa*, *Ricinus communis* and *Vicia faba* sucrose transport protein amino acid sequences.

BLAST scores and probabilities indicate that the instant nucleic acid fragments encode entire or portions of proteins. These sequences represent the first corn, soybean and wheat, amino acid sequences and a new rice sequence encoding sucrose transport proteins.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding a sucrose transport protein in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain

reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below.

Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a sucrose transport protein, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al., (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant sucrose transport proteins in transformed soybean. The

phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising a sequence encoding a sucrose transport protein. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with fluorescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Kline et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the sucrose transport protein and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the

supernatant removed. The DNA-coated particles are then washed once in 400 μ L 70% ethanol and resuspended in 40 μ L of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μ L of the DNA-coated gold particles are then loaded on each macro carrier disk.

5 Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the
10 retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post
15 bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or
20 regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant sucrose transport proteins can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al.
25 (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then,
30 the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve
35 GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 μ g/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μ L of water.

Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the sucrose transport protein are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding all or a substantial portion of a sucrose transport protein comprising a member selected from the group consisting of:

- 5 (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24;
- 10 (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

2. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23.

3. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.

4. A transformed host cell comprising the chimeric gene of Claim 3.

20 5. A sucrose transport protein polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24.

6. A method of altering the level of expression of a sucrose transport protein in a host cell comprising:

- 25 (a) transforming a host cell with the chimeric gene of Claim 3; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of a sucrose transport protein in the transformed host cell.

30 7. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a sucrose transport protein comprising:

- (a) probing a cDNA or genomic library with the nucleic acid fragment of Claim 1;
- 35 (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of Claim 1;
- (c) isolating the DNA clone identified in step (b); and
- (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding a sucrose transport protein.

8. A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding a sucrose transport protein comprising:

- 5 (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23; and
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences
- 10 of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding a sucrose transport protein.

9. The product of the method of Claim 7.

10. The product of the method of Claim 8.

15

TITLE

SUCROSE TRANSPORTERS

ABSTRACT OF THE DISCLOSURE

This invention relates to an isolated nucleic acid fragment encoding a sucrose
transport protein. The invention also relates to the construction of a chimeric gene
encoding all or a portion of the sucrose transport protein, in sense or antisense orientation,
wherein expression of the chimeric gene results in production of altered levels of the
sucrose transport protein in a transformed host cell.

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TMR/dmm

Figure 1

SEQ ID NO:25	(gi 2969887)	MAG	1	60
SEQ ID NO:26	(gi 2723471)	MARGSGAGGGGGG		
SEQ ID NO:27	(gi 542020)	MQS		
SEQ ID NO:28	(gi 1935019)	MEP		
SEQ ID NO:2		MARG		
SEQ ID NO:4		HE		
SEQ ID NO:6		AA		
SEQ ID NO:8				
SEQ ID NO:10		MDSAAGGGGLTAIRL		
SEQ ID NO:12		ME		
SEQ ID NO:14		MEP		
SEQ ID NO:16		AR		
SEQ ID NO:18				
SEQ ID NO:20		MARG		
SEQ ID NO:22		MARG		
SEQ ID NO:24				
SEQ ID NO:25	(gi 2969887)	RSRVSLRLILRVASVACGIQFGWALQLSLLTPYVQELGIPHAWSSI		
SEQ ID NO:26	(gi 2723471)	AAPISLGRLLISGMVAGGVQYGMALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:27	(gi 542020)	PNSSPLRKVMVASIAAGIQFGWALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:28	(gi 1935019)	LEPSPLRKIMVVASIAAGVQFGWALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:2		VAPISLGRLLIAGMVAGGVQYGMALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:4				
SEQ ID NO:6		VAPISLGRLLIAGMVAGGVQYGMALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:8				
SEQ ID NO:10		ARTTTTRKLVACMVAAGVQFGWALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:12		PGPSPLRKMLVSSMAAGIQFGWALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:14		PEASPLRKIMVVASIAAGVQFGWALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:16				
SEQ ID NO:18				
SEQ ID NO:20		---ISLGRLLIAGMVAGGVQYGMALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:22		---ISLGRLLIAGMVAGGVQYGMALQLSLLTPYVQTLGLSHALT		
SEQ ID NO:24		MKGAPKWRVVIACMVAAGVQFGWALQLSLLTPYVQTLGLSHALT		

241
 SEQ ID NO:25 (gi 2969887) AYSGWYKVFPPFLTSSTINCANLKSIFYIDIIIFIITTTYSISAAKERPRISSQDGP-- 300
 SEQ ID NO:26 (gi 2723471) STNNWHKWFPELTKTRACCEACANLKGAFVAVIFLSCLVITLIFAKEVPFKGNA--
 SEQ ID NO:27 (gi 542020) AYTHLYKLFPTKTACDVYCANLKSOFISIVLLSLTVALSYVKEKWPSPDQAVD--
 SEQ ID NO:28 (gi 1935019) AYSKLYHVFPFTKACNVYCANLKSOFISIALTLVATSALIYVKEKTALTPEKTV--
 SEQ ID NO:2 STNNWHKWFPELTKTSACCEACANLKGAFVAVVFLVCLTVTLIFAKEVPYRANEN--
 SEQ ID NO:4 -----
 SEQ ID NO:6 STNNWHKWFPELMTNACCEACANLKGAFVAVVFLIMCLTITLFFAKEVPYRGNQN--
 SEQ ID NO:8 AYSGWYKIFPFTVTPSCSISCANFKSAFLDDIILVVTTCTIVASV-----QEPQ--
 SEQ ID NO:10 ASGNWHKWFPELMTTRACCEACSNLKAFLVAVVFLFCMSVTLYFAEEIPLPTDAQRLS
 SEQ ID NO:12 SYDGLHRLFPEFTEACNVFCANLKSOFFAIVLLVLTTLVLTIVKETPYTPKAKE--
 SEQ ID NO:14 SYGLHNVPFTKTKACDVYCANLKSOFISIALLLTSLTALTIVVKEKTVSSEKTVR--
 SEQ ID NO:16 -----
 SEQ ID NO:18 -----
 SEQ ID NO:20 STNNWHKWFPELTKTRACCEACANLKGAFVAVLAFCLVITVIFAKEIPYKAIAP--
 SEQ ID NO:22 STNNWHKWFPELTKTRACCEACANLKGAFVAVLAFCLVITVIFAKEIPYKAIAP--
 SEQ ID NO:24 ASGNWHKWFPELMTTRACCEACGNLKAFLIAVFLVFLFCMAVTLYFAEEIPLPKDAQQLS

301
 SEQ ID NO:25 (gi 2969887) -----QFSEDGTAQSGHIEEA---F--- 360
 SEQ ID NO:26 (gi 2723471) -----LPT-KSNEPAEPEGT-----
 SEQ ID NO:27 (gi 542020) -----NAEDDTASQASSAQMPFF--
 SEQ ID NO:28 (gi 1935019) -----TT-EDGSSGG-----MPCF--
 SEQ ID NO:2 LPTTKAGGEVETEPT-----G
 SEQ ID NO:4 -----
 SEQ ID NO:6 -----LPT-KANGEVETEPS-----G
 SEQ ID NO:8 -----SFGSDEADHPSTEQEA---F---
 SEQ ID NO:10 DSAPLLNGSRDDNNASNEPRNGALPNGHTDG--SNVPANSNAE-DSNSNRENVEVNDG
 SEQ ID NO:12 -----TDAEKT-----HFSCF--
 SEQ ID NO:14 -----SSVEEDGSHGG-----MPCF--
 SEQ ID NO:16 -----
 SEQ ID NO:18 -----
 SEQ ID NO:20 LPT-KNGQVEVEPT-----G
 SEQ ID NO:22 LPT-KANGQVEVEPT-----G
 SEQ ID NO:24 DSAPLLNGSRDDHDASSEQTNGGLSNGHADA--NHVSANSADAGSNSNKDDVEAFNDG

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SEQ ID NO:27	(gi 542020)	---	---GELLGAFKNLKRPMWILLVTCNLWIA	WFPFLLFDTDMWGREYGGDSSGSAEQL
SEQ ID NO:28	(gi 1935019)	---	---GQLSGAFKELKRPMWILLVTCNLWIA	WFPFLLFDTDMWGKEYYGGTVGEGHA--
SEQ ID NO:2		P---	---LAVLKGFKDLPPGMPSVLLVTAITWL	SWPFFILYDTDMWGREIYHGDPKGSNAQI
SEQ ID NO:4		---	---	---
SEQ ID NO:6		P---	---LAVLKGFKNLPTGMPSVLLVLTGLTWL	SWPFFILYDTDMWGREIYHGDPKGSNAQI
SEQ ID NO:8		---	---LWELFGSFYFTLPVMMVLIIVALTWIG	WEPFILLFDTDMWGRIYRGSPDDPSITQ
SEQ ID NO:10		PGAVL	VNLTSMRHLPPGMYSVLLVMALTWL	SWPFFFLFDTDMWGREIYHGDPNGNLSER
SEQ ID NO:12		---	---CGELCLAFKGLKRPMWMLLVTAVNWIA	WFPFYFLFDTDMWGREYGGDVQ---
SEQ ID NO:14		---	---GQLFGAFRELKRPMWILLVTCNLWD	CLVPFLLFDTDWD---
SEQ ID NO:16		---	---	---
SEQ ID NO:18		---	---AGMPSVLLVLTGLTWL	SWPFFILYDTDMWGRIYHGDPKGTDPDEA
SEQ ID NO:20		P---	---LAVFKGFKNLPP--MPSVLLVLTGLTWL	SWPFFILYDTDMWGRIYHGDPKGTDPDE
SEQ ID NO:22		P---	---LAVFKGFKNLPPGMPSVLLVLTGLTWL	SWPFFILYDTDMWGRIYHGDPKGTDPDEA
SEQ ID NO:24		PGAVL	VKILTSMRHLPPGMYSVLLVMALTWL	SWPFFFLFDTDMWGRIYHGDPKGNASER

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SEQ ID NO:27	(gi 542020)	--KLYDRGVRAGALGLMNSVVLGFTSLGVEVLARGVGGVKRLG	IVN	FLAVCLAMTVL
SEQ ID NO:28	(gi 1935019)	----YDMGVREGALGLMNSVVLGATSLGVDILARGVGGVKRLG	IVN	FLLAICLGLTVL
SEQ ID NO:2		--SAFNEGVRVGA	FGLLNSVILGFSSFLIEPCRKVGP-RVVVWTSN	FMWCVAMAAT
SEQ ID NO:4		----		----
SEQ ID NO:6		--SAFDEGVRVGS	FGLLNSIVLGFSSFLIEPCRKVGP-RVVVWTSN	FMWCVAMAAT
SEQ ID NO:8		S---	YHDGVRMGSGFLMNSVVLGFTSIVLEKLCRKWGAG-LVWGVS	NILMALCF-VAML
SEQ ID NO:10		--KAYDNGVREGAFGLLNSVVLGIGSFVDPLCRMGA-RLVWAI	SNFTVFICMLATAI	
SEQ ID NO:12		--KAYDSGVHAGSLGIMLNAVVLAVMSLAI	EPLGRVVGGIKWLGIWNILLAI	CLGMTVL
SEQ ID NO:14		-----	-----WGVR-----	-----CTE-----
SEQ ID NO:16		-----	-----LGGVKRLWGINFLLAVCLAMTVV	
SEQ ID NO:18		--NAFQAGVRAGAFGLLNSVVLGFSSFLIEPLCKRLGP-RVVWVS	SNFLVCISMAAIC	
SEQ ID NO:20		--ANAFQAGVRAGAFGLLNSVVLGFSSFLIEPLCKRLGP-PRVVWVS	SNFLVCLSMAAIC	
SEQ ID NO:22		--NAFQAGVRAGAFGLLNSVVLGFSSFLIEPLCKRLGP-RVVWVS	SNFLVCLSMAAIC	
SEQ ID NO:24		--KAYDDGVREGAFGLLNSVVLGIGSFLLDPLCRMIGA-RLVWAI	SNFIVFACMLATTI	

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SEQ ID NO:27	(gi 542020)		VTQAESTRRFAVSGGAKPLPPSGVKAGALALFVGMGPQAITYSIPFALASIFNST	
SEQ ID NO:28	(gi 1935019)		VTKLAQHSRQYAPGTGALGDPLPSSEGIKAGALTLSVLGVLPLAITYSIPFALASIFSST	
SEQ ID NO:2			ISFWSLRDYH-----GYVQDAITANASIKAVCLVLF AFLGVLPLAILYSVPFVAVTAQLAAT	
SEQ ID NO:4			LSWISSDLYS-----SKLHHIIGANKTVKITALVVFSLGLPLSITYSVFSTVTAELTAG	
SEQ ID NO:6			ISFWSLKDYH-----GYVQDAITASTSIKAVCLVLF AFLGVLPLAILYSVPFVAVTAQLAAT	
SEQ ID NO:8			VITYVAKNDYPPSGV-----PPTGIVIASLVFTTILGAPLAITYSIPIYAMAASRVEN	
SEQ ID NO:10			LSWISFDLYS-----SKLHHIIGANKTVKNSALIVFSLGLPLSITYSVFSTVTAELTAG	
SEQ ID NO:12			ITKIAEHERLNPALVGN-----PSLGIVKVGSMVFFSVLGIPLAITFSVPFALASIYST	
SEQ ID NO:14			-GKXGER-KGYDKG-----	
SEQ ID NO:16			VTKMADSERQKTLPDGSKTALPPGDIKAGALSIFAVLGAPLAVTFSVPCALASIFSNS	
SEQ ID NO:18			ISWWATQDLH-----GYIQHAITASKEIKIVSLALFAFLGVLPLAILYSVPFVAVTAQLAAN	
SEQ ID NO:20			IISWWATQDL-----HGYIQHAITASKEIKIVSLALFAFLGVLPLAILYSVPFVAVTAQLAA	
SEQ ID NO:22			ISWWATQDLH-----GYIQHAITASKEIKIVSLALFAFLGVLPLAILYSVTFAVTAQLAAN	
SEQ ID NO:24			LSWISYDLYS-----SKLQHVIGADKTVKTSALILFSLGLPLSITYSVFSTVTAELTAG	
		541		600
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SEQ ID NO:27	(gi 542020)		SGAGQGLSLGVNLISIVIPQMIVSVAAGPWDALFGGGNLPAFVVGAVAAALASGIFALTML	
SEQ ID NO:28	(gi 1935019)		SGAGQGLSLGVNLNLAIVIPQMFVSVLSGPWDALFGGGNLPAFVVGAVAAALASGILSIILL	
SEQ ID NO:2			RGGGQGLCTGVNLISIVIPQVIIALGAGPWDALFGKGNIPAFGVASAFALVGGVGVFLL	
SEQ ID NO:4			TGGGQGLATGVNLNLAIVVPQVIVSLGAGPWDALYGGGNTPAFVIAVSFSLAAGVLAVKL	
SEQ ID NO:6			KGGGQGLCTGVNLISIVIPQVIIALGAGPWDALFGKGNIPAFGVASGFALIGGVGVFLL	
SEQ ID NO:8			LGLGQGLAMGILNLAIVIPQVIVSLGSGPWDQLFGGGNAPAFVAAAASFIGGLVAILGL	
SEQ ID NO:10			TGGGQGLATGVNLNLAIVVPQVIVSLGAGPWDALFGGGNVPFALASVSLGAGVLAVKL	
SEQ ID NO:12			SGAGQGLSLGVNLNLAIVVPQVIVSTISGPWDALFGGGNLPAFVLGAVAAVVSAILAVILL	
SEQ ID NO:14			-----	
SEQ ID NO:16			SGAGQGLSLGVNLNLAIVIPQMFVSVLSGPWDALFGGGNLPAFVVGAI SAAVSGILSFTML	
SEQ ID NO:18			RGGGQGLCTGVNLNLAIVIPQVIIAGAGPWDELFGKGNIPAFGVASAFALIGGIVGIFLL	
SEQ ID NO:20			KRGGGQGLCTGVNLNLAIVIPQVIIAGAGPWDELFGKGNIPAFGMASAFALIGGIVGIFL	
SEQ ID NO:22			RCGGQWLCTGVNLNLAIVIPQVIIAGAGPWDELFGKGNIPAFGVASAFALIGGIVGIFL	
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Hitz, William D.
Rafalski, J. Antoni

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Arg Leu Ile Leu Ala Gly Met Val Ala Gly Gly Val Gln Tyr Gly Trp
          35          40          45

Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val Gln Thr Leu Gly Leu
 50          55          60

Ser His Ala Leu Thr Ser Phe Met Trp Leu Cys Gly Pro Ile Ala Gly
 65          70          75          80

Leu Val Val Gln Pro Leu Val Gly Leu Tyr Ser Asp Arg Cys Thr Ser
          85          90          95

Arg Trp Gly Arg Arg Arg Pro Phe Ile Leu Thr Gly Cys Met Leu Ile
          100          105          110

Cys Val Ala Val Ile Val Val Gly Phe Ser Ser Asp Ile Gly Ala Ala
          115          120          125

Leu Gly Asp Thr Lys Glu His Cys Ser Leu Tyr His Gly Pro Arg Trp
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His Ala Ala Ile Val Tyr Val Leu Gly Phe Trp Leu Leu Asp Phe Ser
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Asn Asn Thr Val Gln Gly Pro Ala Arg Ala Met Met Ala Asp Leu Cys
          165          170          175

Asp His His Gly Pro Ser Ala Ala Asn Ser Ile Phe Cys Ser Trp Met
          180          185          190

Ala Leu Gly Asn Ile Leu Gly Tyr Ser Ser Gly Ser Thr Asn Asn Trp
          195          200          205

His Lys Trp Phe Pro Phe Leu Lys Thr Ser Ala Cys Cys Glu Ala Cys
          210          215          220

Ala Asn Leu Lys Gly Ala Phe Leu Val Ala Val Val Phe Leu Val Leu
          225          230          235          240

Cys Leu Thr Val Thr Leu Ile Phe Ala Lys Glu Val Pro Tyr Arg Ala
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Asn Glu Asn Leu Pro Thr Thr Lys Ala Gly Gly Glu Val Glu Thr Glu
          260          265          270

Pro Thr Gly Pro Leu Ala Val Leu Lys Gly Phe Lys Asp Leu Pro Pro
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Gly Met Pro Ser Val Leu Leu Val Thr Ala Ile Thr Trp Leu Ser Trp
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 340 345 350
 Gly Phe Ser Ser Phe Leu Ile Glu Pro Met Cys Arg Lys Val Gly Pro
 355 360 365
 Arg Val Val Trp Val Thr Ser Asn Phe Met Val Cys Val Ala Met Ala
 370 375 380
 Ala Thr Ala Leu Ile Ser Phe Trp Ser Leu Arg Asp Tyr His Gly Tyr
 385 390 395 400
 Val Gln Asp Ala Ile Thr Ala Asn Ala Ser Ile Lys Ala Val Cys Leu
 405 410 415
 Val Leu Phe Ala Phe Leu Gly Val Pro Leu Ala Ile Leu Tyr Ser Val
 420 425 430
 Pro Phe Ala Val Thr Ala Gln Leu Ala Ala Thr Arg Gly Gly Gly Gln
 435 440 445
 Gly Leu Cys Thr Gly Val Leu Asn Ile Ser Ile Val Ile Pro Gln Val
 450 455 460
 Ile Ile Ala Leu Gly Ala Gly Pro Trp Asp Ala Leu Phe Gly Lys Gly
 465 470 475 480
 Asn Ile Pro Ala Phe Gly Val Ala Ser Ala Phe Ala Leu Val Gly Gly
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 35 40 45
 Ser Val Thr Ala Glu Leu Thr Ala Gly Thr Gly Gly Gly Gln Gly Leu
 50 55 60
 Ala Thr Gly Val Leu Asn Leu Ala Ile Val Val Pro Gln Ile Val Val
 65 70 75 80
 Ser Leu Gly Ala Gly Pro Trp Asp Ala Leu Tyr Gly Gly Gly Asn Thr
 85 90 95
 Pro Ala Phe Val Leu Ala Ser Val Phe Ser Leu Ala Ala Gly Val Leu
 100 105 110
 Ala Val Leu Lys Leu Pro Lys Leu Ser Asn Ser Tyr Gln Ser Ala Gly
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Leu Leu Thr Pro Tyr Val Gln Thr Leu Gly Leu Ser His Ala Leu Thr
          35          40          45

Ser Phe Met Trp Leu Cys Gly Pro Ile Ala Gly Leu Val Val Gln Pro
          50          55          60

Leu Val Gly Leu Tyr Ser Asp Arg Cys Thr Ala Arg Trp Gly Arg Arg
          65          70          75          80

Arg Pro Phe Ile Leu Ile Gly Cys Met Leu Ile Cys Leu Ala Val Ile
          85          90          95

Val Val Gly Phe Ser Ser Asp Ile Gly Ala Ala Leu Gly Asp Thr Lys
          100          105          110

Glu His Cys Ser Leu Tyr His Gly Pro Arg Trp His Ala Ala Ile Val
          115          120          125

Tyr Val Leu Gly Phe Trp Leu Leu Asp Phe Ser Asn Asn Thr Val Gln
          130          135          140

Gly Pro Ala Arg Ala Met Met Ala Asp Leu Cys Gly His His Gly Pro
          145          150          155          160

Ser Ala Ala Asn Ser Ile Phe Cys Ser Trp Met Ala Leu Gly Asn Ile
          165          170          175

Leu Gly Tyr Ser Ser Gly Ser Thr Asn Asn Trp His Lys Trp Phe Pro
          180          185          190

Phe Leu Met Thr Asn Ala Cys Cys Glu Ala Cys Ala Asn Leu Lys Gly
          195          200          205

Ala Phe Leu Val Ala Val Val Phe Leu Ile Met Cys Leu Thr Ile Thr
          210          215          220

Leu Phe Phe Ala Lys Glu Val Pro Tyr Arg Gly Asn Gln Asn Leu Pro
          225          230          235          240

Thr Lys Ala Asn Gly Glu Val Glu Thr Glu Pro Ser Gly Pro Leu Ala
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Val Leu Lys Gly Phe Lys Asn Leu Pro Thr Gly Met Pro Ser Val Leu
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Leu Val Thr Gly Leu Thr Trp Leu Ser Trp Phe Pro Phe Ile Leu Tyr
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Asp Thr Asp Trp Met Gly Arg Glu Ile Tyr His Gly Asp Pro Lys Gly
290 295 300

Ser Asn Ala Gln Ile Ser Ala Phe Asp Glu Gly Val Arg Val Gly Ser
305 310 315 320

Phe Gly Leu Leu Leu Asn Ser Ile Val Leu Gly Phe Ser Ser Phe Leu
325 330 335

Ile Glu Pro Met Cys Arg Lys Val Gly Pro Arg Val Val Trp Val Thr
340 345 350

Ser Asn Phe Met Val Cys Val Ala Met Ala Ala Thr Ala Leu Ile Ser
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Phe Trp Ser Leu Lys Asp Tyr His Gly Tyr Val Gln Asp Ala Ile Thr
370 375 380

Ala Ser Thr Ser Ile Lys Ala Val Cys Leu Val Leu Phe Ala Phe Leu
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Gly Val Pro Leu Ala Ile Leu Tyr Ser Val Pro Phe Ala Val Thr Ala
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Gln Leu Ala Ala Thr Lys Gly Gly Gly Gln Gly Leu Cys Thr Gly Val
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Leu Asn Ile Ser Ile Val Ile Pro Gln Val Ile Ile Ala Leu Gly Ala
435 440 445

Gly Pro Trp Asp Ala Leu Phe Gly Lys Gly Asn Ile Pro Ala Phe Gly
450 455 460

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<210> 8
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<212> PRT
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Thr Arg Leu Gly Ala Ile Ile Val Tyr Leu Val Gly Phe Trp Leu Leu
      35             40             45

Asp Val Gly Asn Asn Ala Thr Gln Gly Pro Cys Arg Ala Phe Leu Ala
      50             55             60

Asp Leu Thr Glu Asn Asp Pro Arg Arg Thr Arg Ile Ala Asn Ala Tyr
      65             70             75             80

Phe Ser Leu Phe Met Ala Leu Gly Asn Ile Leu Gly Tyr Ala Thr Gly
      85             90             95

Ala Tyr Ser Gly Trp Tyr Lys Ile Phe Pro Phe Thr Val Thr Pro Ser
      100            105            110

Cys Ser Ile Ser Cys Ala Asn Phe Lys Ser Ala Phe Leu Leu Asp Ile
      115            120            125

Ile Ile Leu Val Val Thr Thr Cys Ile Thr Val Ala Ser Val Gln Glu
      130            135            140

Pro Gln Ser Phe Gly Ser Asp Glu Ala Asp His Pro Ser Thr Glu Gln
      145            150            155            160

Glu Ala Phe Leu Trp Glu Leu Phe Gly Ser Phe Arg Tyr Phe Thr Leu
      165            170            175

Pro Val Trp Met Val Leu Ile Val Thr Ala Leu Thr Trp Ile Gly Trp
      180            185            190

Phe Pro Phe Ile Leu Phe Asp Thr Asp Trp Met Gly Arg Glu Ile Tyr
      195            200            205

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 225 230 240
 Phe Thr Ser Ile Val Leu Glu Lys Leu Cys Arg Lys Trp Gly Ala Gly
 245 250 255
 Leu Val Trp Gly Val Ser Asn Ile Leu Met Ala Leu Cys Phe Val Ala
 260 265 270
 Met Leu Val Ile Thr Tyr Val Ala Lys Asn Met Asp Tyr Pro Pro Ser
 275 280 285
 Gly Val Pro Pro Thr Gly Ile Val Ile Ala Ser Leu Val Val Phe Thr
 290 295 300
 Ile Leu Gly Ala Pro Leu Ala Ile Thr Tyr Ser Ile Pro Tyr Ala Met
 305 310 315 320
 Ala Ala Ser Arg Val Glu Asn Leu Gly Leu Gly Gln Gly Leu Ala Met
 325 330 335
 Gly Ile Leu Asn Leu Ala Ile Val Ile Pro Gln Val Ile Val Ser Leu
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 Gly Ser Gly Pro Trp Asp Gln Leu Phe Gly Gly Gly Asn Ala Pro Ala
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 <212> DNA
 <213> *Oryza sativa*

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 gacctattac tggttttgtg gttcaaccat gtgttggtgt ctggagtgc aaatgccgtt 600
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<210> 10
<211> 667
<212> PRT
<213> Oryza sativa

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Lys His Thr Thr Arg Thr Gln Gln Gln Gly Arg Arg Gln Phe Pro Ile
      20              25              30

Leu Pro Arg Pro Ala Ser Pro Arg Leu Ser Leu Thr Leu Gln Thr Pro
      35              40              45

Thr Ser Asp Ala Ala Ser Leu Ala Pro Cys Pro Arg Arg Ser His Gln
      50              55              60

Thr Leu Pro Asp Leu Arg Pro Ala Met Asp Ser Ala Ala Gly Gly Gly
      65              70              75              80

Gly Leu Thr Ala Ile Arg Leu Pro Tyr Arg His Leu Arg Asp Ala Glu
      85              90              95

Met Glu Leu Val Ser Leu Asn Gly Gly Thr Pro Arg Gly Gly Ser Pro
      100             105             110

Lys Asp Pro Asp Ala Thr His Gln Gln Gly Pro Pro Ala Ala Arg Thr
      115             120             125

Thr Thr Thr Arg Lys Leu Val Leu Ala Cys Met Val Ala Ala Gly Val
      130             135             140

Gln Phe Gly Trp Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Ile Gln
      145             150             155             160

Thr Leu Gly Ile Asp His Ala Met Ala Ser Phe Ile Trp Leu Cys Gly
      165             170             175

Pro Ile Thr Gly Phe Val Val Gln Pro Cys Val Gly Val Trp Ser Asp
      180             185             190

Lys Cys Arg Ser Lys Tyr Gly Arg Arg Arg Pro Phe Ile Leu Ala Gly
      195             200             205

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Cys	Leu	Met	Ile	Cys	Phe	Ala	Val	Thr	Leu	Ile	Gly	Phe	Ser	Ala	Asp	210	215	220
Leu	Gly	Tyr	Ile	Leu	Gly	Asp	Thr	Thr	Glu	His	Cys	Ser	Thr	Tyr	Lys	225	230	235
Gly	Ser	Arg	Phe	Arg	Ala	Ala	Ile	Ile	Phe	Val	Leu	Gly	Phe	Trp	Met	245	250	255
Leu	Asp	Leu	Ala	Asn	Asn	Thr	Val	Gln	Gly	Pro	Ala	Arg	Ala	Leu	Leu	260	265	270
Ala	Asp	Leu	Ser	Gly	Pro	Asp	Gln	Cys	Asn	Ser	Ala	Asn	Ala	Ile	Phe	275	280	285
Cys	Thr	Trp	Met	Ala	Val	Gly	Asn	Val	Leu	Gly	Phe	Ser	Ser	Gly	Ala	290	295	300
Ser	Gly	Asn	Trp	His	Lys	Trp	Phe	Pro	Phe	Leu	Met	Thr	Arg	Ala	Cys	305	310	315
Cys	Glu	Ala	Cys	Ser	Asn	Leu	Lys	Ala	Ala	Phe	Leu	Val	Ala	Val	Val	325	330	335
Phe	Leu	Leu	Phe	Cys	Met	Ser	Val	Thr	Leu	Tyr	Phe	Ala	Glu	Glu	Ile	340	345	350
Pro	Leu	Glu	Pro	Thr	Asp	Ala	Gln	Arg	Leu	Ser	Asp	Ser	Ala	Pro	Leu	355	360	365
Leu	Asn	Gly	Ser	Arg	Asp	Asp	Asn	Asn	Ala	Ser	Asn	Glu	Pro	Arg	Asn	370	375	380
Gly	Ala	Leu	Pro	Asn	Gly	His	Thr	Asp	Gly	Ser	Asn	Val	Pro	Ala	Asn	385	390	395
Ser	Asn	Ala	Glu	Asp	Ser	Asn	Ser	Asn	Arg	Glu	Asn	Val	Glu	Val	Phe	405	410	415
Asn	Asp	Gly	Pro	Gly	Ala	Val	Leu	Val	Asn	Ile	Leu	Thr	Ser	Met	Arg	420	425	430
His	Leu	Pro	Pro	Gly	Met	Tyr	Ser	Val	Leu	Leu	Val	Met	Ala	Leu	Thr	435	440	445
Trp	Leu	Ser	Trp	Phe	Pro	Phe	Phe	Leu	Phe	Asp	Thr	Asp	Trp	Met	Gly	450	455	460
Arg	Glu	Val	Tyr	His	Gly	Asp	Pro	Asn	Gly	Asn	Leu	Ser	Glu	Arg	Lys	465	470	475
Ala	Tyr	Asp	Asn	Gly	Val	Arg	Glu	Gly	Ala	Phe	Gly	Leu	Leu	Leu	Asn	485	490	495
Ser	Val	Val	Leu	Gly	Ile	Gly	Ser	Phe	Leu	Val	Asp	Pro	Leu	Cys	Arg	500	505	510
Leu	Met	Gly	Ala	Arg	Leu	Val	Trp	Ala	Ile	Ser	Asn	Phe	Thr	Val	Phe	515	520	525
Ile	Cys	Met	Leu	Ala	Thr	Ala	Ile	Leu	Ser	Trp	Ile	Ser	Phe	Asp	Leu	530	535	540

Tyr Ser Ser Lys Leu His His Ile Ile Gly Ala Asn Lys Thr Val Lys
 545 550 555 560
 Asn Ser Ala Leu Ile Val Phe Ser Leu Leu Gly Leu Pro Leu Ser Ile
 565 570 575
 Thr Tyr Ser Val Pro Phe Ser Val Thr Ala Glu Leu Thr Ala Gly Thr
 580 585 590
 Gly Gly Gly Gln Gly Leu Ala Thr Gly Val Leu Asn Leu Ala Ile Val
 595 600 605
 Val Pro Gln Ile Val Val Ser Leu Gly Ala Gly Pro Trp Asp Ala Leu
 610 615 620
 Phe Gly Gly Gly Asn Val Pro Ala Phe Ala Leu Ala Ser Val Phe Ser
 625 630 635 640
 Leu Gly Ala Gly Val Leu Ala Val Leu Lys Leu Pro Lys Leu Pro Asn
 645 650 655
 Ser Tyr Arg Ser Ala Gly Phe His Gly Phe Gly
 660 665

<210> 11
 <211> 1885
 <212> DNA
 <213> Glycine max

<400> 11
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 gacaacctga cccaaaagac tcggccacgt gcagtggcga tcttcgtgat cgggttttgg 480
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 gctgcccgggg atgagaaaaa gacaaaggca gccaatgcct tcttctcttt cttcatggcc 600
 gtcggcaaca tcctgggcta tgctgcggga tctacgacg gcctccaccg cctcttcccc 660
 ttcacggaaa ccgaggcatg caacgtcttc tgcgcaaacc tcaagagttg cttcttcttc 720
 gctatcgtcc tcctgggtgt cctcaccacc ttggtgctga ttacogtgaa agaaactccc 780
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 tgccggagaac tttgtcttgc attcaagggg ctgaagaggc caatgtggat gttgatgttg 900
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 gttcttctgc tgccaactcc aaagaaagct gatgaggtca gggcttctag cctcaacatg 1560
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<210> 12
 <211> 494
 <212> PRT
 <213> Glycine max

<400> 12
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 20 25 30
 Ser Leu Leu Thr Pro Tyr Val Gln Thr Leu Gly Val Pro His Ala Trp
 35 40 45
 Ala Ser Phe Ile Trp Leu Cys Gly Pro Ile Ser Gly Leu Leu Val Gln
 50 55 60
 Pro Ile Val Gly Tyr Ser Ser Asp Arg Cys Gln Ser Arg Phe Gly Arg
 65 70 75 80
 Arg Arg Pro Phe Ile Leu Ala Gly Ser Leu Ala Val Ala Ile Ala Val
 85 90 95
 Phe Leu Ile Gly Tyr Ala Ala Asp Ile Gly His Ala Ala Gly Asp Asn
 100 105 110
 Leu Thr Gln Lys Thr Arg Pro Arg Ala Val Ala Ile Phe Val Ile Gly
 115 120 125
 Phe Trp Ile Leu Asp Val Ala Asn Asn Met Leu Gln Gly Pro Cys Arg
 130 135 140
 Ala Phe Leu Gly Asp Leu Ala Ala Gly Asp Glu Lys Lys Thr Lys Ala
 145 150 155 160
 Ala Asn Ala Phe Phe Ser Phe Phe Met Ala Val Gly Asn Ile Leu Gly
 165 170 175
 Tyr Ala Ala Gly Ser Tyr Asp Gly Leu His Arg Leu Phe Pro Phe Thr
 180 185 190
 Glu Thr Glu Ala Cys Asn Val Phe Cys Ala Asn Leu Lys Ser Cys Phe
 195 200 205
 Phe Phe Ala Ile Val Leu Leu Val Val Leu Thr Thr Leu Val Leu Ile
 210 215 220
 Thr Val Lys Glu Thr Pro Tyr Thr Pro Lys Ala Glu Lys Glu Thr Glu
 225 230 235 240
 Asp Ala Glu Lys Thr His Phe Ser Cys Phe Cys Gly Glu Leu Cys Leu
 245 250 255
 Ala Phe Lys Gly Leu Lys Arg Pro Met Trp Met Leu Met Leu Val Thr
 260 265 270
 Ala Val Asn Trp Ile Ala Trp Phe Pro Tyr Phe Leu Phe Asp Thr Asp
 275 280 285
 Trp Met Gly Arg Glu Val Tyr Gly Gly Asp Val Gly Gln Lys Ala Tyr
 290 295 300

Asp Ser Gly Val His Ala Gly Ser Leu Gly Leu Met Leu Asn Ala Val
 305 310 315 320
 Val Leu Ala Val Met Ser Leu Ala Ile Glu Pro Leu Gly Arg Val Val
 325 330 335
 Gly Gly Ile Lys Trp Leu Trp Gly Ile Val Asn Ile Leu Leu Ala Ile
 340 345 350
 Cys Leu Gly Met Thr Val Leu Ile Thr Lys Ile Ala Glu His Glu Arg
 355 360 365
 Leu Leu Asn Pro Ala Leu Val Gly Asn Pro Ser Leu Gly Ile Lys Val
 370 375 380
 Gly Ser Met Val Phe Phe Ser Val Leu Gly Ile Pro Leu Ala Ile Thr
 385 390 395 400
 Phe Ser Val Pro Phe Ala Leu Ala Ser Ile Tyr Ser Ser Thr Ser Gly
 405 410 415
 Ala Gly Gln Gly Leu Ser Leu Gly Val Leu Asn Ile Ala Ile Val Val
 420 425 430
 Pro Gln Met Ile Val Ser Thr Ile Ser Gly Pro Trp Asp Ala Leu Phe
 435 440 445
 Gly Gly Gly Asn Leu Pro Ala Phe Val Leu Gly Ala Val Ala Ala Val
 450 455 460
 Val Ser Ala Ile Leu Ala Val Leu Leu Leu Pro Thr Pro Lys Lys Ala
 465 470 475 480
 Asp Glu Val Arg Ala Ser Ser Leu Asn Met Gly Ser Leu His
 485 490

<210> 13
 <211> 1041
 <212> DNA
 <213> Glycine max

<220>
 <221> unsure
 <222> (1007)

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 cggaggccag tcccctccgg aagatcatgg tgggtggcctc catcgccgcc ggggtgcaat 180
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 ggtacgataa ggggttccgt t 1041

<210> 14
 <211> 322
 <212> PRT
 <213> Glycine max

<220>
 <221> UNSURE
 <222> (311)

<220>
 <221> UNSURE
 <222> (321)

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 Ser Ser Leu His Thr Glu Ala Pro Pro Glu Ala Ser Pro Leu Arg
 20 25 30
 Lys Ile Met Val Val Ala Ser Ile Ala Ala Gly Val Gln Phe Gly Trp
 35 40 45
 Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val Gln Leu Leu Gly Ile
 50 55 60
 Pro His Thr Trp Ala Ala Phe Ile Trp Leu Cys Gly Pro Ile Ser Gly
 65 70 75 80
 Met Leu Val Gln Pro Ile Val Gly Tyr His Ser Asp Arg Cys Thr Ser
 85 90 95
 Arg Phe Gly Arg Arg Arg Pro Phe Ile Ala Ala Gly Ser Leu Ala Val
 100 105 110
 Ala Ile Ala Val Phe Leu Ile Gly Tyr Ala Ala Asp Leu Gly His Met
 115 120 125
 Phe Gly Asp Ser Leu Ala Lys Lys Thr Ala Pro Arg His Arg Ile Phe
 130 135 140
 Val Val Gly Phe Trp Ile Leu Asp Val Ala Asn Asn Met Leu Gln Gly
 145 150 155 160
 Pro Cys Arg Ala Leu Leu Gly Asp Leu Cys Ala Gly Glu Gln Arg Lys
 165 170 175
 Thr Arg Asn Ala Asn Ala Phe Phe Ser Phe Phe Met Ala Val Gly Asn
 180 185 190
 Val Leu Gly Tyr Ala Ala Gly Ser Tyr Ser Gly Leu His Asn Val Phe
 195 200 205
 Pro Phe Thr Lys Thr Lys Ala Cys Asp Val Tyr Cys Ala Asn Leu Lys
 210 215 220
 Ser Cys Phe Phe Leu Ser Ile Ala Leu Leu Leu Thr Leu Ser Thr Ile
 225 230 235 240
 Ala Leu Thr Tyr Val Lys Glu Lys Thr Val Ser Ser Glu Lys Thr Val
 245 250 255

Arg Ser Ser Val Glu Glu Asp Gly Ser His Gly Gly Met Pro Cys Phe
260 265 270

Gly Gln Leu Phe Gly Ala Phe Arg Glu Leu Lys Arg Pro Met Trp Ile
275 280 285

Leu Leu Leu Val Thr Cys Leu Asn Trp Asp Cys Leu Val Pro Phe Leu
290 295 300

Leu Phe Asp Thr Asp Trp Xaa Gly Arg Glu Val Tyr Gly Gly Lys Ile
305 310 315 320

Xaa Gly

<210> 15
<211> 578
<212> DNA
<213> Vernonia mespilifolia

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gtttgttttg ccatgacggt ggtggtgacc aaaatggcag actctgaacg acagtttaag 120
acgttgcccg acggtagcaa aaccgcgttg ccaccaggcg gcgacattaa agccggtgct 180
ttgtcaattt ttgccgtcct cgggtgcccc ctagctgtga ctttcagtgt tccatgtgct 240
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aatctagcaa tcgtcatacc acagatgttc gtatcagtac taagtggacc atgggacgca 360
ctgttcggcg gtggaaactt accagcattt gtggttgagg caatttcggc tgcagtaagt 420
gggatattat cgttcaccat gcttccttcg ccacccccag atgtcgtact ttcaaagggt 480
tccggagggtg ggatgcatta gagagtaaata aactgccact caacacgtcc cgattgtgtc 540
agattgggac atttaggacc aaaaaaaaaa aaaaaaaaaa 578

<210> 16
<211> 166
<212> PRT
<213> Vernonia mespilifolia

<400> 16
Ala Arg Gly Trp Leu Gly Gly Val Lys Arg Leu Trp Gly Gly Ile Asn
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Phe Leu Leu Ala Val Cys Leu Ala Met Thr Val Val Val Thr Lys Met
20 25 30

Ala Asp Ser Glu Arg Gln Phe Lys Thr Leu Pro Asp Gly Ser Lys Thr
35 40 45

Ala Leu Pro Pro Gly Gly Asp Ile Lys Ala Gly Ala Leu Ser Ile Phe
50 55 60

Ala Val Leu Gly Ala Pro Leu Ala Val Thr Phe Ser Val Pro Cys Ala
65 70 75 80

Leu Ala Ser Ile Phe Ser Asn Ser Ser Gly Ala Gly Gln Gly Leu Ser
85 90 95

Leu Gly Val Leu Asn Leu Ala Ile Val Ile Pro Gln Met Phe Val Ser
100 105 110

Val Leu Ser Gly Pro Trp Asp Ala Leu Phe Gly Gly Gly Asn Leu Pro
115 120 125

Ala Phe Val Val Gly Ala Ile Ser Ala Ala Val Ser Gly Ile Leu Ser
130 135 140

Phe Thr Met Leu Pro Ser Pro Pro Pro Asp Val Val Leu Ser Lys Val
 145 150 155 160

Ser Gly Gly Gly Met His
 165

<210> 17
 <211> 1062
 <212> DNA
 <213> Triticum aestivum

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 ccgacgaggc caacgcgttc caggcagggtg tcaggggcggg ggcgttcggc ctgctactca 180
 actcgggtcgt cctgggggttc agctcgttcc tgatcgagcc gctgtgcaag aggctaggcc 240
 cgcggttggt gtgggtgtca agcaacttcc tcgtctgcat ctccatggcc gccatttgca 300
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 ttctgtacag tgtccctttc gcggtgacgg cgcagctggc ggcgaacaga ggcggtggcc 480
 aagggtctgt cacgggcgtg ctgaacatcg ccatcgtgat accccagggtg atcatcgcg 540
 tggggggcggg gccgtgggac gagctgttcg gcaagggcaa catcccggcg ttcggcggtg 600
 cgtccgcctt cgcgctcatc ggcgcatcg tcggcatatt cctgctgccc aagatctcca 660
 ggcgccagtt ccgggcgtc agcggcgcg gtcactgacc ggcgcgcgcg ccggtcggcc 720
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 aaattttcgc ataggcgtaa ctagggggt ctcgcctaag gactccgtag agcagaataa 840
 gaattgtgag gaacctgtat gtgtgtgtc tgtatgtgcg tgtaagtcag tgcgtgtagc 900
 ggaaaatgga cagaggaatg cgggcatcca tcgccggctg ggggtgtcgtc tttgggttgt 960
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 cagcgacaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 1062

<210> 18
 <211> 232
 <212> PRT
 <213> Triticum aestivum

<400> 18
 Ala Gly Met Pro Ser Val Leu Leu Val Thr Gly Leu Thr Trp Leu Ser
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 Trp Phe Pro Phe Ile Leu Tyr Asp Thr Asp Trp Met Gly Arg Glu Ile
 20 25 30
 Tyr His Gly Asp Pro Lys Gly Thr Pro Asp Glu Ala Asn Ala Phe Gln
 35 40 45
 Ala Gly Val Arg Ala Gly Ala Phe Gly Leu Leu Leu Asn Ser Val Val
 50 55 60
 Leu Gly Phe Ser Ser Phe Leu Ile Glu Pro Leu Cys Lys Arg Leu Gly
 65 70 75 80
 Pro Arg Val Val Trp Val Ser Ser Asn Phe Leu Val Cys Ile Ser Met
 85 90 95
 Ala Ala Ile Cys Ile Ile Ser Trp Trp Ala Thr Gln Asp Leu His Gly
 100 105 110
 Tyr Ile Gln His Ala Ile Thr Ala Ser Lys Glu Ile Lys Ile Val Ser
 115 120 125
 Leu Ala Leu Phe Ala Phe Leu Gly Ile Pro Leu Ala Ile Leu Tyr Ser
 130 135 140

Val Pro Phe Ala Val Thr Ala Gln Leu Ala Ala Asn Arg Gly Gly Gly
 145 150 155 160

Gln Gly Leu Cys Thr Gly Val Leu Asn Ile Ala Ile Val Ile Pro Gln
 165 170 175

Val Ile Ile Ala Val Gly Ala Gly Pro Trp Asp Glu Leu Phe Gly Lys
 180 185 190

Gly Asn Ile Pro Ala Phe Gly Val Ala Ser Ala Phe Ala Leu Ile Gly
 195 200 205

Gly Ile Val Gly Ile Phe Leu Leu Pro Lys Ile Ser Arg Arg Gln Phe
 210 215 220

Arg Ala Val Ser Gly Gly Gly His
 225 230

<210> 19
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 <212> DNA
 <213> Triticum aestivum

<400> 19
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 gacgaggtgg tgatcgccag ggcggcctct ctgccatggc gcgcggcgga ggcaacggcg 240
 aggtggagct ctcggtcggg gtcggcgcg gaggcgcgcg cgcgcgcggc gggggggagc 300
 aaccgcgcgt ggacatcagc ctcggcagac tcatcctcgc cggcatggtc gccggcgcg 360
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 tttcgcatgc tctgacttca ttcatgtggc tctgcgcccc tattgctgga ttagtggttc 480
 aaccatgcgt tgggctctac agtgacaagt gcacatctag atggggaaga cgcagaccgt 540
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 acattggagc tggctctgggt gacagcaagg aagagtgcag tctctatcat gggcctcgtt 660
 ggcacgctgc aattgtgtat gttcttggat tctggctcct tgacttctcc aacaacactg 720
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 ctctcgccat tctgtacagt gtccctttcg cggtgacggc gcagctggcg gcgaagagag 1560
 gcggtggcca aggcgtgtgc acgggcgtgc tcaacatcgc catcgtgata cccaggtga 1620
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 cggaggtccc agcccagccc gccatttacc aaattttcgc ataggcgtaa ctagggtggt 1860
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 tgtatgtgcg tgtaagtcag tgcgtgtagc ggaaaatgga cagaggaatg tgggcatcca 1980
 tcaccggctg ggggtgtcgtc tttgggttgt gacttgtgtg tagcaaacca aggtgatcaa 2040
 gtgaggggaa atgaatggat gatgaacttt cagcgacaaa aaa 2083

<210> 20
 <211> 522

<212> PRT

<213> Triticum aestivum

<400> 20

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Gly Gly Gly Gly Gly Gly Ala Ala Gly Gly Gly Glu Gln Pro Ala Val
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Asp Ile Ser Leu Gly Arg Leu Ile Leu Ala Gly Met Val Ala Gly Gly
35 40 45
Val Gln Tyr Gly Trp Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val
50 55 60
Gln Thr Leu Gly Leu Ser His Ala Leu Thr Ser Phe Met Trp Leu Cys
65 70 75 80
Gly Pro Ile Ala Gly Leu Val Val Gln Pro Cys Val Gly Leu Tyr Ser
85 90 95
Asp Lys Cys Thr Ser Arg Trp Gly Arg Arg Arg Pro Phe Ile Leu Thr
100 105 110
Gly Cys Ile Leu Ile Cys Ile Ala Val Val Val Val Gly Phe Ser Ala
115 120 125
Asp Ile Gly Ala Gly Leu Gly Asp Ser Lys Glu Glu Cys Ser Leu Tyr
130 135 140
His Gly Pro Arg Trp His Ala Ala Ile Val Tyr Val Leu Gly Phe Trp
145 150 155 160
Leu Leu Asp Phe Ser Asn Asn Thr Val Gln Gly Pro Ala Arg Ala Leu
165 170 175
Met Ala Asp Leu Ser Ala Gln His Gly Pro Ser Ala Ala Asn Ser Ile
180 185 190
Phe Cys Ser Trp Met Ala Leu Gly Asn Ile Leu Gly Tyr Ser Ser Gly
195 200 205
Ser Thr Asn Asn Trp His Lys Trp Phe Pro Phe Leu Arg Thr Arg Ala
210 215 220
Cys Cys Glu Ala Cys Ala Asn Leu Lys Gly Ala Phe Leu Val Ala Val
225 230 235 240
Leu Val Leu Ala Phe Cys Leu Val Ile Thr Val Ile Phe Ala Lys Glu
245 250 255
Ile Pro Tyr Lys Ala Ile Ala Pro Leu Pro Thr Lys Gly Asn Gly Gln
260 265 270
Val Glu Val Glu Pro Thr Gly Pro Leu Ala Val Phe Lys Gly Phe Lys
275 280 285
Asn Leu Pro Pro Met Pro Ser Val Leu Leu Val Thr Gly Leu Thr Trp
290 295 300
Leu Ser Trp Phe Pro Phe Ile Leu Tyr Asp Thr Asp Trp Met Gly Arg
305 310 315 320

Glu Ile Tyr His Gly Asp Pro Lys Gly Thr Pro Asp Glu Ala Asn Ala
 325 330 335
 Phe Gln Ala Gly Val Arg Ala Gly Ala Phe Gly Leu Leu Leu Asn Ser
 340 345 350
 Val Val Leu Gly Phe Ser Ser Phe Leu Ile Glu Pro Leu Cys Lys Arg
 355 360 365
 Leu Gly Pro Arg Val Val Trp Val Ser Ser Asn Phe Leu Val Cys Leu
 370 375 380
 Ser Met Ala Ala Ile Cys Ile Ile Ser Trp Trp Ala Thr Gln Asp Leu
 385 390 395 400
 His Gly Tyr Ile Gln His Ala Ile Thr Ala Ser Lys Glu Ile Lys Ile
 405 410 415
 Val Ser Leu Ala Leu Phe Ala Phe Leu Gly Ile Pro Leu Ala Ile Leu
 420 425 430
 Tyr Ser Val Pro Phe Ala Val Thr Ala Gln Leu Ala Ala Lys Arg Gly
 435 440 445
 Gly Gly Gln Gly Leu Cys Thr Gly Val Leu Asn Ile Ala Ile Val Ile
 450 455 460
 Pro Gln Val Ile Ile Ala Val Gly Ala Gly Pro Trp Asp Glu Leu Phe
 465 470 475 480
 Gly Lys Gly Asn Ile Pro Ala Phe Gly Met Ala Ser Ala Phe Ala Leu
 485 490 495
 Ile Gly Gly Ile Val Gly Ile Phe Leu Leu Pro Lys Ile Ser Arg Arg
 500 505 510
 Gln Phe Arg Ala Val Ser Gly Gly Gly His
 515 520

<210> 21
 <211> 2160
 <212> DNA
 <213> Triticum aestivum

<400> 21
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 ccggcggttg tccgacgcgc cgtagagttg ataggcgaac gaacggggcg gtgatcgtcc 180
 gggcgggccc cctgcgacga tggcgcgcg cggcggcaac ggcgaggtgg agctctcggt 240
 gggggtcggc ggaggcggcg ccggcgccgg cggggcggac gccccgcgcg tggacatcag 300
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 ggagataccg tacaaggcga ttgcgcccct cccaacaaag gccaatggcc aggttgaagt 1020
 cgagcccacc gggccgctcg ccgtcttcaa aggccttcaag aacttgctc ctggaatgcc 1080
 gtcagtgtc ctgctcacc gcctcacctg gctgtcctgg tccccctca tctgtacga 1140

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tgtcactttc gccgtgacgg cgcagctggc ggcgaaacaga tgcgggtgggc aatggctgtg 1560
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gccgtgggac gagctgttcg gcaagggcaa catcccggcg ttcggcgtgg cgtccgcctt 1680
cgcgctcatc ggcgcatcg tcggcatatt cctgctgccc aagatctcca ggctccagtt 1740
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gaaggccgat cgcgcgggcc cgaaggtccc agcccagctc ggcatttacc aaattttcgc 1860
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cagaggaatg cgggcatcca tcgcccggctg ggggtgtcgtc tttgggttgt gacttgtgtg 2040
tagcaaacca aggtgatcaa gtgaggggaa aagaatggat gatgaacttt cagcgacaaa 2100
aaaaaaaaa aaaaaaaaaa aaaaaataa aaaaaaaaaa aagaaaaaaa taaaaaaaaa 2160

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<210> 22
<211> 522
<212> PRT
<213> Triticum aestivum

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<400> 22
Met Ala Arg Gly Gly Gly Asn Gly Glu Val Glu Leu Ser Val Gly Val
  1             5             10             15

Gly Gly Gly Gly Ala Gly Ala Gly Gly Ala Asp Ala Pro Ala Val Asp
      20             25             30

Ile Ser Leu Gly Arg Leu Ile Leu Ala Gly Met Val Ala Gly Gly Val
      35             40             45

Gln Tyr Gly Trp Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val Gln
      50             55             60

Thr Leu Gly Leu Ser His Ala Leu Thr Ser Phe Met Trp Leu Cys Gly
      65             70             75             80

Pro Ile Ala Gly Leu Val Val Gln Pro Cys Val Gly Leu Tyr Ser Asp
      85             90             95

Lys Cys Thr Ser Arg Trp Gly Arg Arg Arg Pro Phe Ile Leu Thr Gly
      100            105            110

Cys Ile Leu Ile Cys Ile Ala Val Val Val Val Gly Phe Ser Ala Asp
      115            120            125

Ile Gly Ala Ala Leu Gly Asp Ser Lys Glu Glu Cys Ser Leu Tyr His
      130            135            140

Gly Pro Arg Trp His Ala Ala Ile Val Tyr Val Leu Gly Phe Trp Leu
      145            150            155            160

Leu Asp Phe Ser Asn Asn Thr Val Gln Gly Pro Ala Arg Ala Leu Met
      165            170            175

Ala Asp Leu Ser Ala Gln His Gly Pro Ser Ala Ala Asn Ser Ile Phe
      180            185            190

Cys Ser Trp Met Ala Leu Gly Asn Ile Leu Gly Tyr Ser Ser Gly Ser
      195            200            205

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Thr Asn Asn Trp His Lys Trp Phe Pro Phe Leu Arg Thr Arg Ala Cys
 210 215 220
 Cys Glu Ala Cys Ala Asn Leu Lys Gly Ala Phe Leu Val Ala Val Leu
 225 230 235 240
 Phe Leu Ala Phe Cys Leu Val Ile Thr Val Ile Phe Ala Lys Glu Ile
 245 250 255
 Pro Tyr Lys Ala Ile Ala Pro Leu Pro Thr Lys Ala Asn Gly Gln Val
 260 265 270
 Glu Val Glu Pro Thr Gly Pro Leu Ala Val Phe Lys Gly Phe Lys Asn
 275 280 285
 Leu Pro Pro Gly Met Pro Ser Val Leu Leu Val Thr Gly Leu Thr Trp
 290 295 300
 Leu Ser Trp Phe Pro Phe Ile Leu Tyr Asp Thr Asp Trp Met Gly Arg
 305 310 315 320
 Glu Ile Tyr His Gly Asp Pro Lys Gly Thr Pro Asp Glu Ala Asn Ala
 325 330 335
 Phe Gln Ala Gly Val Arg Ala Gly Ala Phe Gly Leu Leu Leu Asn Ser
 340 345 350
 Val Val Leu Gly Phe Ser Ser Phe Leu Ile Glu Pro Leu Cys Lys Arg
 355 360 365
 Leu Gly Pro Arg Val Val Trp Val Ser Ser Asn Phe Leu Val Cys Leu
 370 375 380
 Ser Met Ala Ala Ile Cys Ile Ile Ser Trp Trp Ala Thr Gln Asp Leu
 385 390 395 400
 His Gly Tyr Ile Gln His Ala Ile Thr Ala Ser Lys Glu Ile Lys Ile
 405 410 415
 Val Ser Leu Ala Leu Phe Ala Phe Leu Gly Ile Pro Leu Ala Ile Leu
 420 425 430
 Tyr Ser Val Thr Phe Ala Val Thr Ala Gln Leu Ala Ala Asn Arg Cys
 435 440 445
 Gly Gly Gln Trp Leu Cys Thr Gly Val Leu Asn Ile Ala Ile Ala Ile
 450 455 460
 Pro Gln Val Ile Ile Ala Leu Gly Ala Gly Pro Trp Asp Glu Leu Phe
 465 470 475 480
 Gly Lys Gly Asn Ile Pro Ala Phe Gly Val Ala Ser Ala Phe Ala Leu
 485 490 495
 Ile Gly Gly Ile Val Gly Ile Phe Leu Leu Pro Lys Ile Ser Arg Leu
 500 505 510
 Gln Phe Arg Ala Val Ser Gly Gly Gly His
 515 520

<210> 23
 <211> 2030
 <212> DNA
 <213> Triticum aestivum

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<400> 23
cggaagcgac gccgcgcggc ccaaggagga acagggcagc ggcgcggggg cgggggaagg 60
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gcagttcggc tgggcgctcc agctctccct cctcaccccc tacatccaga ctctaggaat 180
agaccatgcc atggcgctcc tcatttggtt ttgcgggccc attactggtt ttgtggttca 240
accgtgtggt ggtgtctgga gtgacaagtg ccgctccaag tacgggagga gacggccgtt 300
cattttggct ggatgcgtgc tgatttgtgc agctgtaact ttagtcgggt tttctgcaga 360
ccttggttac atgttaggag acaccactga gcactgcagt acatacaaag gtctacgata 420
tcgagctgct tttattttca tttttggatt ctggatgctg gaccttgcaa ataatacagt 480
tcaaggacct gctcgtgccc tcctagctga tctttcaggt cccgatcaat gtaattcggc 540
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gagtgggaat tggcacaagt ggtttccttt tctgatgact agggcctgtt gtgaagcttg 660
tggtaatatt aaagcagctt tcttgattgc agttgtattc cttctgtttt gcatggctgt 720
taccctctac tttgctgaag agattccact ggaaccaaag gatgcacagc agttatctga 780
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ctctttcctt atcgatccat tatgccgat gattggtgca agattgggtt gggcaatcag 1260
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tcttgctaga tacacagtta ataagactac agatcagata gactaggata aagagatagt 1860
ttttaggcct gtgtgcatac aagtgtcgat gagaagttgt aaaacatgta cactgttttt 1920
ttgtactgta tatgtagtga aatttcatag atggccggat gtgttctggt ccgataaaaa 1980
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2030

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<210> 24
<211> 563
<212> PRT
<213> Triticum aestivum

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<400> 24
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 1             5             10             15

Ala Gly Glu Gly Gly Met Lys Gly Ala Pro Lys Trp Arg Val Val Leu
 20             25             30

Ala Cys Met Val Ala Ala Gly Val Gln Phe Gly Trp Ala Leu Gln Leu
 35             40             45

Ser Leu Leu Thr Pro Tyr Ile Gln Thr Leu Gly Ile Asp His Ala Met
 50             55             60

Ala Ser Phe Ile Trp Leu Cys Gly Pro Ile Thr Gly Phe Val Val Gln
 65             70             75             80

Pro Cys Val Gly Val Trp Ser Asp Lys Cys Arg Ser Lys Tyr Gly Arg
 85             90             95

Arg Arg Pro Phe Ile Leu Ala Gly Cys Val Leu Ile Cys Ala Ala Val
100             105             110

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Thr	Leu	Val	Gly	Phe	Ser	Ala	Asp	Leu	Gly	Tyr	Met	Leu	Gly	Asp	Thr	115	120	125
Thr	Glu	His	Cys	Ser	Thr	Tyr	Lys	Gly	Leu	Arg	Tyr	Arg	Ala	Ala	Phe	130	135	140
Ile	Phe	Ile	Phe	Gly	Phe	Trp	Met	Leu	Asp	Leu	Ala	Asn	Asn	Thr	Val	145	150	155
Gln	Gly	Pro	Ala	Arg	Ala	Leu	Leu	Ala	Asp	Leu	Ser	Gly	Pro	Asp	Gln	165	170	175
Cys	Asn	Ser	Ala	Asn	Ala	Ile	Phe	Cys	Ser	Trp	Met	Ala	Val	Gly	Asn	180	185	190
Val	Leu	Gly	Phe	Ser	Ala	Gly	Ala	Ser	Gly	Asn	Trp	His	Lys	Trp	Phe	195	200	205
Pro	Phe	Leu	Met	Thr	Arg	Ala	Cys	Cys	Glu	Ala	Cys	Gly	Asn	Leu	Lys	210	215	220
Ala	Ala	Phe	Leu	Ile	Ala	Val	Val	Phe	Leu	Leu	Phe	Cys	Met	Ala	Val	225	230	235
Thr	Leu	Tyr	Phe	Ala	Glu	Glu	Ile	Pro	Leu	Glu	Pro	Lys	Asp	Ala	Gln	245	250	255
Gln	Leu	Ser	Asp	Ser	Ala	Pro	Leu	Leu	Asn	Gly	Ser	Arg	Asp	Asp	His	260	265	270
Asp	Ala	Ser	Ser	Glu	Gln	Thr	Asn	Gly	Gly	Leu	Ser	Asn	Gly	His	Ala	275	280	285
Asp	Ala	Asn	His	Val	Ser	Ala	Asn	Ser	Ser	Ala	Asp	Ala	Gly	Ser	Asn	290	295	300
Ser	Asn	Lys	Asp	Asp	Val	Glu	Ala	Phe	Asn	Asp	Gly	Pro	Gly	Ala	Val	305	310	315
Leu	Val	Lys	Ile	Leu	Thr	Ser	Met	Arg	His	Leu	Pro	Pro	Gly	Met	Tyr	325	330	335
Ser	Val	Leu	Leu	Val	Met	Ala	Leu	Thr	Trp	Leu	Ser	Trp	Phe	Pro	Phe	340	345	350
Phe	Leu	Phe	Asp	Thr	Asp	Trp	Met	Gly	Arg	Glu	Val	Tyr	His	Gly	Asp	355	360	365
Pro	Lys	Gly	Asn	Ala	Ser	Glu	Arg	Lys	Ala	Tyr	Asp	Asp	Gly	Val	Arg	370	375	380
Glu	Gly	Ala	Phe	Gly	Leu	Leu	Leu	Asn	Ser	Val	Val	Leu	Gly	Ile	Gly	385	390	395
Ser	Phe	Leu	Ile	Asp	Pro	Leu	Cys	Arg	Met	Ile	Gly	Ala	Arg	Leu	Val	405	410	415
Trp	Ala	Ile	Ser	Asn	Phe	Ile	Val	Phe	Ala	Cys	Met	Leu	Ala	Thr	Thr	420	425	430
Ile	Leu	Ser	Trp	Ile	Ser	Tyr	Asp	Leu	Tyr	Ser	Ser	Lys	Leu	Gln	His	435	440	445

Ile Val Gly Ala Asp Lys Thr Val Lys Thr Ser Ala Leu Ile Leu Phe
 450 455 460
 Ser Leu Leu Gly Leu Pro Leu Ser Ile Thr Tyr Ser Val Pro Phe Ser
 465 470 475 480
 Val Thr Ala Glu Leu Thr Ala Gly Thr Gly Gly Gly Gln Gly Leu Ala
 485 490 495
 Thr Gly Val Leu Asn Leu Ala Ile Val Ala Pro Gln Ile Val Val Ser
 500 505 510
 Leu Gly Ala Gly Pro Trp Asp Lys Leu Leu Gly Gly Gly Asn Val Pro
 515 520 525
 Ala Phe Ala Leu Ala Ser Val Phe Ser Leu Ala Ala Gly Val Leu Ala
 530 535 540
 Val Ile Lys Leu Pro Lys Leu Ser Asn Asn Tyr Gln Ser Ala Gly Phe
 545 550 555 560
 His Met Gly

<210> 25

<211> 501

<212> PRT

<213> Daucus carota

<400> 25

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 1 5 10 15
 Ala Ala Pro Pro Pro Arg Ser Arg Val Ser Leu Arg Leu Leu Leu Arg
 20 25 30
 Val Ala Ser Val Ala Cys Gly Ile Gln Phe Gly Trp Ala Leu Gln Leu
 35 40 45
 Ser Leu Leu Thr Pro Tyr Val Gln Glu Leu Gly Ile Pro His Ala Trp
 50 55 60
 Ser Ser Ile Ile Trp Leu Cys Gly Pro Leu Ser Gly Leu Leu Val Gln
 65 70 75 80
 Pro Ile Val Gly His Met Ser Asp Gln Cys Thr Ser Lys Tyr Gly Arg
 85 90 95
 Arg Arg Pro Phe Ile Val Ala Gly Gly Thr Ala Ile Ile Leu Ala Val
 100 105 110
 Ile Ile Ile Ala His Ser Ala Asp Ile Gly Gly Leu Leu Gly Asp Thr
 115 120 125
 Ala Asp Asn Lys Thr Met Ala Ile Val Ala Phe Val Ile Gly Phe Trp
 130 135 140
 Ile Leu Asp Val Ala Asn Asn Met Thr Gln Gly Pro Cys Arg Ala Leu
 145 150 155 160

Leu Ala Asp Leu Thr Gly Asn Asp Ala Arg Arg Thr Arg Val Ala Asn
 165 170 175
 Ala Tyr Phe Ser Leu Phe Met Ala Ile Gly Asn Val Leu Gly Tyr Ala
 180 185 190
 Thr Gly Ala Tyr Ser Gly Trp Tyr Lys Val Phe Pro Phe Ser Leu Thr
 195 200 205
 Ser Ser Cys Thr Ile Asn Cys Ala Asn Leu Lys Ser Ala Phe Tyr Ile
 210 215 220
 Asp Ile Ile Phe Ile Ile Ile Thr Thr Tyr Ile Ser Ile Ser Ala Ala
 225 230 235 240
 Lys Glu Arg Pro Arg Ile Ser Ser Gln Asp Gly Pro Gln Phe Ser Glu
 245 250 255
 Asp Gly Thr Ala Gln Ser Gly His Ile Glu Glu Ala Phe Leu Trp Glu
 260 265 270
 Leu Phe Gly Thr Phe Arg Leu Leu Pro Gly Ser Val Trp Val Ile Leu
 275 280 285
 Leu Val Thr Cys Leu Asn Trp Ile Gly Trp Phe Pro Phe Ile Leu Phe
 290 295 300
 Asp Thr Asp Trp Met Gly Arg Glu Ile Tyr Gly Gly Glu Pro Asn Gln
 305 310 315 320
 Gly Gln Ser Tyr Ser Asp Gly Val Arg Met Gly Ala Phe Gly Leu Met
 325 330 335
 Met Asn Ser Val Val Leu Gly Ile Thr Ser Val Leu Met Glu Lys Leu
 340 345 350
 Cys Arg Ile Trp Gly Ser Gly Phe Met Trp Gly Leu Ser Asn Ile Leu
 355 360 365
 Met Thr Ile Cys Phe Phe Ala Met Leu Leu Ile Thr Phe Ile Ala Lys
 370 375 380
 Asn Met Asp Tyr Gly Thr Asn Pro Pro Pro Asn Gly Ile Val Ile Ser
 385 390 395 400
 Ala Leu Ile Val Phe Ala Ile Leu Gly Ile Pro Leu Ala Ile Thr Tyr
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Ala Gly Met Val Val Gln Pro Cys Val Gly Leu Tyr Ser Asp Arg Cys
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Thr Ser Lys Trp Gly Arg Arg Arg Pro Tyr Ile Leu Thr Gly Cys Val
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 Tyr His Gly Asp Pro Lys Gly Thr Asp Pro Gln Ile Glu Ala Phe Asn
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 Phe Gly Trp Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val Gln Leu
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 Leu Gly Ile His His Thr Trp Ala Ala Tyr Ile Trp Leu Cys Gly Pro
 65 70 75 80
 Ile Ser Gly Met Leu Val Gln Pro Ile Val Gly Tyr His Ser Asp Arg
 85 90 95
 Cys Thr Ser Arg Phe Gly Arg Arg Arg Pro Phe Ile Ala Ala Gly Ser
 100 105 110
 Ile Ala Val Ala Ile Ala Val Phe Leu Ile Gly Tyr Ala Ala Asp Leu
 115 120 125
 Gly His Ser Phe Gly Asp Ser Leu Asp Gln Lys Val Arg Pro Arg Ala
 130 135 140

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EXPRESS MAIL LABEL NO. EL073740966US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

S. ALLEN ET AL.

CASE NO.: BB1162 US NA

APPLICATION NO.: UNKNOWN

GROUP ART UNIT: UNKNOWN

FILED: CONCURRENTLY HEREWITH

EXAMINER: UNKNOWN

FOR: SUCROSE TRANSPORTERS

POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I hereby appoint THOMAS M. RIZZO (Registration No. 41,272) and KENING LI (Registration No. 44,872) the power to prosecute the above-identified application and to transact all business in the Patent and Trademark Office connected herewith.

All other powers are hereby revoked.

Please send all correspondence in such application to the principal attorney of record at the following address:

E. I. du Pont de Nemours & Co.
Legal - Patents
Wilmington, Delaware 19898

Respectfully submitted,

Barbara J. Massie

BARBARA J. MASSIE
Assistant Secretary, Patent Board

Dated: 10/5/2000

**EXPRESS MAIL LABEL NO. EL073740966US
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

IN THE APPLICATION OF:

E. I. DUPONT DE NEMOURS AND COMPANY

CASE NO.: BB1162 US NA

APPLICATION NO.: UNKNOWN

GROUP ART UNIT: UNKNOWN

FILED: CONCURRENTLY HERewith

EXAMINER: UNKNOWN

FOR: **SUCROSE TRANSPORTERS**

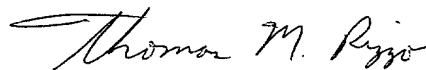
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION IN ACCORDANCE WITH 37 CFR 1.821

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR 1.821(c) and (e), respectively are the same.

Respectfully submitted,



THOMAS M. RIZZO
ATTORNEY FOR APPLICANTS
REGISTRATION NO. 41,272
TELEPHONE: 302-892-7760
FACSIMILE: 302-892-1026

Dated: October 5, 2000